NOTE ON THE NATURE AND SOURCE OF "PURPLE X."[1]

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In the summer of 1913, Dr. O. C. Glaser ('14) found (1) that if a suspension of *Arbacia* sperm be boiled it turns purple, (2) that this purple color disappears if the boiled suspension be allowed to stand over night, and (3) that initiation of development in *Arbacia* eggs, either by sperm or by egg secretion, can be inhibited by the addition to the eggs of this boiled suspension *so long as it is purple.* This purple substance, he provisionally designated as "purple x."

I. Source of "Purple X."

In 1914, while working with various inhibitors of fertilization in *Arbacia*, I wished to use "purple x" and found that it did not always appear when *Arbacia* sperm was boiled. This naturally led to a search for the source of the substance. Sperm was obtained from a large number of males. Each one was rinsed in fresh water, and after the peristome was cut, placed aboral side down in a clean dry watch-glass, until the seminal fluid had been freely shed. A portion of the sperm from each animal was then mixed with filtered sea-water and examined under the microscope and a second, larger portion of each was boiled. It was found that, as a rule, the suspensions which showed fewest foreign cells—blood-cells or fragments of organs—were least likely to form "purple x." Suspensions which appeared, under the microscope, fairly free from foreign cells, could be depended upon to give a colorless filtrate when boiled.

What, then, is the impurity which produces "purple x"? By a series of experiments properly checked and repeated, I was able to eliminate successively, the filtered perivisceral fluid (serum), the blood clot which had been washed in sea-water

[1] From the Marine Biological Laboratory at Woods Hole and the Zoölogical Laboratory of the University of Michigan.

to free it from serum, fresh fluid containing both serum and blood cells, pieces of the alimentary tract, and mesentery. If a piece of fresh mature testis be boiled in sea-water, in sea-water plus sperm, or in distilled water, a purple compound is formed. This color can also be obtained by treating the testis with strong alcohol, which, as observed under the microscope, turns testis cells from greenish brown to purplish. Whether or not this would happen with immature testes could not be determined, since the experiments were carried on during the breeding season.

The sperm from one male, when examined under the microscope, showed the presence of a few small pieces of testis, and, when boiled, or treated with alcohol, turned purple. The testes were then washed in running sea-water for about three hours, during which they were occasionally pinched with forceps to help free them from sperm. When this was apparently all washed out, a portion of the testis was boiled in sea-water, without giving a purple color. Treating with absolute alcohol, boiling in distilled water, and boiling in sea-water plus fresh sperm also failed to bring out any purple tinge from pieces of the washed testes. The rest were then left standing over night in a finger-bowl of sea-water. After twenty-four hours, it was observed that they tinged the water slightly but distinctly purple.

It seems clear, then, that "purple x" arises from the fresh testis, or from a reaction between testis and sperm. It still remains to be learned whether or not it may be obtained from the ovaries also.

II. Chemical Nature of "Purple X."

The best known pigment of sea-urchins is echinochrome, whose chemical reactions are well established. It may be obtained from the red blood cells by laking with distilled water, by extracting with alcohol, chloroform or ether. The neutral extracts are a cherry red, the addition of a small amount of NaOH turns them yellowish, and acidulating with HCl produces a red-yellow color (MacMunn, '85).

Echinochrome was extracted from the washed blood clot by laking with one volume of distilled water. An equal amount of "double sea-water" (sea-water boiled down to one-half its

original volume) was then added so that the salt content might equal that of the solution with which it was to be compared. A series of experiments was then run in duplicate, using this echinochrome on the one hand, and "purple x" on the other. The addition of NaOH and HCl to echinochrome gave the results described by MacMunn. No visible change could be obtained in the "purple x," however, by the addition of either reagent. Thus it was established that, whatever the chemical nature of "purple x" may be, it is not identical with echinochrome.

III. Physiological Effect of Boiled Sperm.

The addition of boiled sperm suspension to eggs, in *Arbacia*, causes the jelly surrounding the egg to swell, as can be demonstrated by putting them into India ink. It also becomes more sticky. As a result of this, the eggs adhere to each other and to the bottom of the dish. When fresh spermatozoa are added, many get caught in the jelly and form "halos," but some are able to penetrate to the eggs, so that fertilization is not inhibited.

However, when "purple x" is present in the boiled sperm, fertilization by fresh sperm and auto-initiation by egg-secretion is inhibited, as shown in the following table. The percentages were all obtained by counting 200 or more eggs, and a vertical column represents eggs from the same female, treated in different ways.

	Per Cent. of Eggs Divided.						
	A	*B*	*C*	*D*	*E*	*F*	*G*
Eggs without sperm	0	0	0	0	0	0	0
Eggs + sperm	84	72	73	72.3	Polyspermy		
Eggs + boiled sperm (colorless) + fresh sperm		68	55	76.7	82		
Eggs + boiled sperm (purple) + fresh sperm	0			26.6	39		
Eggs + egg secretion + hypertonic after-treatment						43	56.9
Eggs + egg secretion + boiled sperm (purple) + hypertonic after-treatment						0	1±

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Literature.

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